Genetic Divergence of the Pest Moth Chloridea virescens (Noctuidae: Heliothinae) Feeding on a Newly Documented Host Plant in the Atacama Desert of Northern Chile

Authors: Santos-Zamorano, Bárbara, Huanca-Mamani, Wilson, and Vargas, Héctor A.

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Additional key words: Asteraceae, *Chloridea subflexa*, *Heliothis subflexa*, *Heliothis virescens*, *Trixis cacalioides*

The polyphagous moth *Chloridea virescens* (Fabricius) (Lepidoptera: Noctuidae: Heliothinae) is a widely distributed pest in the New World, ranging from United States to Argentina (Poole et al. 1993). Along the Pacific coast it reaches the Pacific Northwest as its northern limit, including the states of Oregon and Washington, United States (Landolt 2008), while its southern limit is in the northernmost part of Chile (Parra et al. 1986). The wide host range of *C. virescens* includes a great number of plants, many of which are agricultural crops, belonging to many families (Pogue 2013, Ventura et al. 2016).

It is known that characterization of the genetic variation is important to understanding the biology of insect pests, especially in widely distributed species, because these sometimes exhibit genetic differentiation throughout their geographic ranges, which can be especially interesting to develop pest management strategies at the local level (Salinas-Hernandez & Saldamandano-Benjumea 2011, Diaz-Montilla et al. 2013, Maia et al. 2016, Velasco-Cuervo et al. 2016). Albernaz et al. (2012) assessed the genetic variation of *C. virescens* populations feeding on cotton and soybean in Brazil based on sequence analysis of mitochondrial
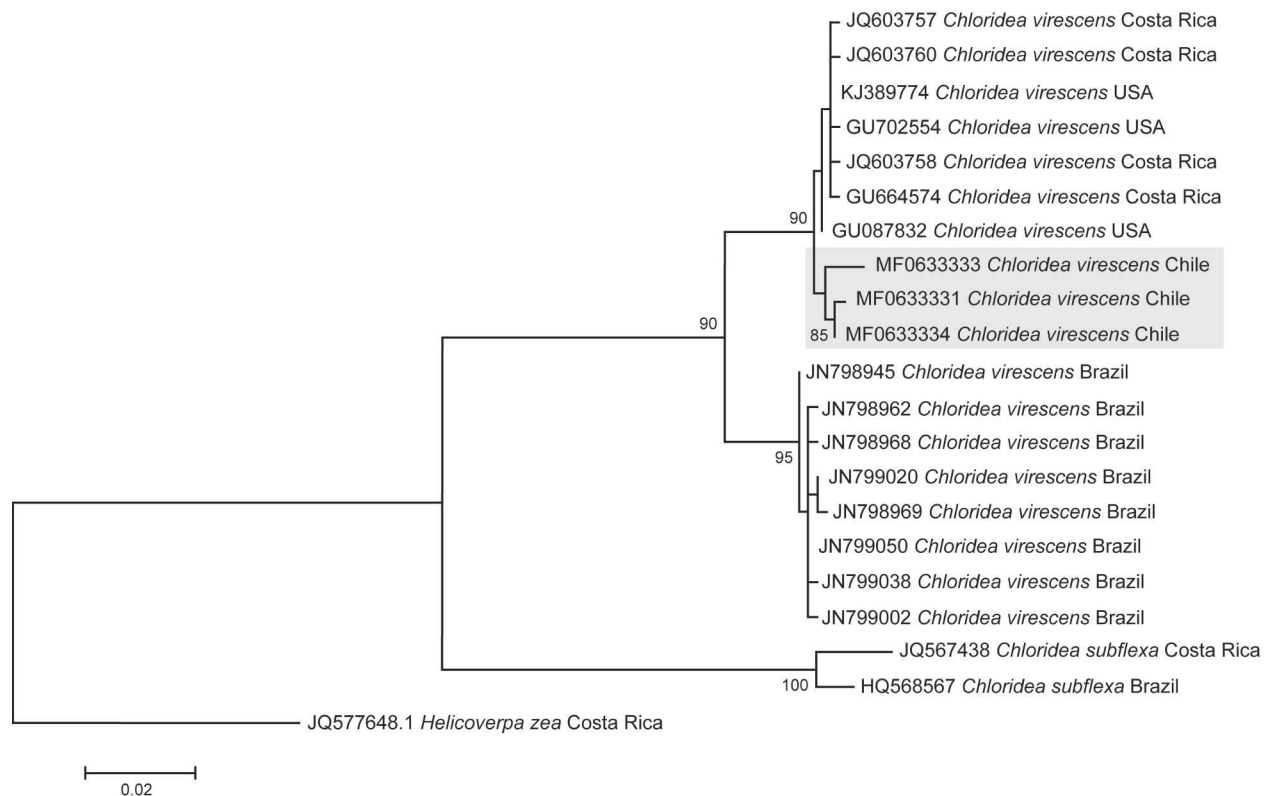

FIG. 1. Maximum likelihood tree of the sequences of the DNA barcode fragment (651 bp) of the cytochrome c oxidase subunit I (COI) gene of *Chloridea virescens* (Lepidoptera. Noctuidae) from different parts of the New World. Gray area indicates the newly reported sequences from the Azapa Valley, Atacama Desert of northern Chile. Bootstrap supports above 70% are shown.

DNA. Interestingly, they found that some haplotypes were exclusive to one geographic region or one host plant, which was interpreted as an initial differentiation of some populations in the Brazilian range of *C. virescens*, a pattern that was mostly corroborated using microsatellites in a later study (Domingues et al. 2013). Subsequently, Mitchell and Gopurenko (2016) analyzed the DNA barcode fragment (sensu Hebert et al. 2003) of the sequences provided by Albernaz et al. (2012) with samples from additional New World sites and found two very distinct clusters, one of which included Central and Northern American haplotypes, while the other only included Brazilian haplotypes.

The narrow Chilean range of *C. virescens* is restricted to the transverse valleys of the Atacama Desert (Parra et al. 1986), where the feeding activity of its larvae is a serious problem for some horticultural crops (Klein-Koch & Waterhouse 2000). We provided the first assessment of the genetic divergence of *C. virescens* of Chile based on analysis of DNA barcode sequences of specimens collected at the larval stage on a newly documented host plant.

Larvae of *C. virescens* were collected on the shrub *Trixis cacalioides* (Kunth) D. Don (Asteraceae) in the Azapa Valley, Arica Province, Atacama Desert of northern Chile in August 2016, and were brought to the laboratory in plastic bags. Some larvae were kept in 95% ethanol at -20 °C for DNA extraction, and the remainder were reared in the laboratory to obtain adults for taxonomic identification based on the examination of the genitalia morphology. Three legs and a bit of thoracic muscles of the adults were removed before mounting and were used for DNA extraction.

Genomic DNA was extracted from larvae and thoracic muscles of the adults following the procedures described in Huanca-Mamani et al. (2015). Amplification and sequencing was performed by Macrogen Inc. (South Korea) using the primers LCO-1490 and HCO-2198 (Folmer et al. 1994). Conditions for the polymerase chain reaction were: 5 min at 94 ºC, 35 cycles of 30 sec at 94 ºC, 30 sec at 47 ºC, 1 min at 72 ºC, and a final elongation step of 10 min at 72 ºC.

The sequences were analyzed in the software MEGA6 (Tamura et al. 2013) following the procedures described by Hall (2013), including sequence alignment by the ClustalW method, estimation of sequence divergence by the Kimura 2-parameter (K2P) method and a Maximum Likelihood (ML) analysis with TN93+G as model of sequence evolution. The bootstrap method (1,000 replicates) was used to assess the statistical support of the nodes. The *C. virescens* sequences analyzed in the study of Mitchell & Gopurenko (2016), which include the Brazilian sequences provided by Albernaz et al.

TABLE 1. Nucleotide variation among haplotypes of the DNA barcode fragment (658 bp) of the cytochrome c oxidase subunit I (COI) gene of *Chloridea virescens* (Lepidoptera: Noctuidae) collected as larvae on *Trixis cacalioides* (Asteraceae) in the Azapa Valley, Atacama Desert of northern Chile.

	Variable sites [a]						n
	217	427	451	542	616	646	
Haplotype							
H1	G	C	T	C	T	T	2
H2	A	T	C	T	C	C	1
H3	-	-	-	-	C	-	1

[a] "-" indicates nucleotide identity to the H1 haplotype.

(2012), were downloaded from BOLD (Ratnasingham & Hebert 2007), and the software DnaSp (Librado & Rozas 2009) was subsequently used to select one sequence of each haplotype to be included in the ML analysis. As the Brazilian barcode sequences were 651 base pairs (bp) length, this was the minimal size for sequence selection. Sequences of the only congeneric species available in BOLD (*C. subflexa* Guenée) were also included in the analysis, and one sequence of *Helicoverpa zea* (Boddie) was used to root the tree, as *Helicoverpa* Hardwick is close to *Chloridea* Duncan & Westwood (Pogue 2013).

Four sequences of 658 bp of the DNA barcode fragment were obtained from the Chilean samples of *C. virescens*, with mean nucleotide composition 40.2% T, 14.7% C, 30.9% A, and 14.2% G. Six variable sites were detected, with all the mutations of the transition type (Table 1). The variation determined the presence of three haplotypes, H1 represented by two individuals (GenBank accession: MF063331, MF063332) and H2 and H3 by one individual each (MF063333 and MF063334, respectively). The genetic distance was 0.2–0.9% (K2P) among the Chilean haplotypes, 0.5–0.9% with *C. virescens* from Central and North America, 2.4–3.0% with *C. virescens* from Brazil, and 5.8–6.6% with the congeneric *C. subflexa*.

The alignment for the ML analysis included 21 DNA barcode sequences (Table 2) of 651 bp with 74 variable sites, 45 of which were parsimony informative. All the *C. virescens* sequences were clustered in a well-supported group (Fig. 1). Furthermore, in accordance with the results reported by Mitchell & Gopurenko (2016), this group was in turn internally divided in two also well-supported subgroups, one of which included only the Brazilian haplotypes, while the three samples from Chile

TABLE 2. DNA barcode sequences used in the maximum likelihood analysis.

Species	BOLD accession	GenBank accession	Length (bp)	Country
Chloridea virescens	---	MF063331	658	Chile
Chloridea virescens	---	MF063333	658	Chile
Chloridea virescens	---	MF063334	658	Chile
Chloridea virescens	GBMIN30031-13	JN799050	651	Brazil
Chloridea virescens	GBMIN30037-13	JN799038	651	Brazil
Chloridea virescens	GBMIN30046-13	JN799020	651	Brazil
Chloridea virescens	GBMIN30055-13	JN799002	651	Brazil
Chloridea virescens	GBMIN30075-13	JN798962	651	Brazil
Chloridea virescens	GBMIN30072-13	JN798968	651	Brazil
Chloridea virescens	GBMIN30128-13	JN798969	651	Brazil
Chloridea virescens	GBMIN30140-13	JN798945	651	Brazil
Chloridea virescens	BBLOD1268-11	KJ389774	658	USA
Chloridea virescens	HELNA527-09	GU702554	658	USA
Chloridea virescens	LOT347-04	GU087832	658	USA
Chloridea virescens	MHMXF822-07	JQ603757	658	Costa Rica
Chloridea virescens	MHMXF823-07	JQ603758	658	Costa Rica
Chloridea virescens	MHMXF825-07	JQ603760	658	Costa Rica
Chloridea virescens	MHMXZ853-09	GU664574	658	Costa Rica
Chloridea subflexa	BLPBH617-07	JQ567438	658	Costa Rica
Chloridea subflexa	INCTA907-10	HQ568567	658	Brazil
Helicoverpa zea	BLPAA705-06	JQ577648.1	658	Costa Rica

were clustered with the Central and North American haplotypes. However, the internal relationships of the two subgroups of *C. virescens* were not well resolved.

The K2P distances and the result of the ML analysis suggest that the Chilean representatives of *C. virescens* are more closely related to Central and North American moths than to those of Brazil. It is probable that despite the recognized high dispersal power of the adults of *C. virescens* (Hernández & Blanco 2010) the Andes Cordillera has been an effective barrier for this moth in South America. As a consequence, gene flow would be possible mostly between populations on the same side of the Andes, enhancing the genetic divergence of the populations belonging to the eastern and western sides of these mountains. Similarly some mitochondrial haplotypes of *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) are restricted to specific geographic areas separated by the Andes (Díaz-Montilla et al. 2013). Indeed, the uplift of the Andes has been also described as an important factor underlying the diversification of other Neotropical groups of Lepidoptera (Brower 1994, Massardo et al. 2015).

As already highlighted by Mitchell & Gopurenko (2016), the divergence level of the Brazilian with Central and North American haplotypes is in the range generally recognized as interspecific for Lepidoptera (Hebert et al. 2003, Hausmann et al. 2011). Indeed Mitchell & Gopurenko (2016) suggested that the Brazilian populations could have been misidentified as *C. virescens*. Although this issue is beyond the scope of our

work, the results also show high divergence between the Chilean and Brazilian haplotypes (2.4–3.0% K2P). In contrast, the divergence of the Chilean and Central and North American haplotypes (0.5–0.9% K2P) is in the range generally recognized as intra-specific for Lepidoptera (Hebert et al., 2003; Hausmann et al. 2011). In addition, the high support of the two internal clusters of *C. virescens* and their reciprocally monophyletic status highlight the necessity of further examination of the genetic differentiation of *C. virescens* throughout its range using a multi-locus approach in order to assess cryptic speciation.

Most pest species of Heliothinae are highly polyphagous (Cunningham & Zalucki 2014). Although *C. virescens* is a polyphagous pest using several hosts of the family Asteraceae, this is the first time that *T. cacalioides* is reported as its host plant. This finding has at least two important consequences. First, as the knowledge of alternative host plants is useful for the understanding of the biology of the insect pests at a local scale (Abney et al. 2007, Albernaz et al. 2012, Domingues et al. 2013), this newly recorded interaction should be considered by local farmers for planning pest management practices. It should be interesting, for example, to assess the role of *T. cacalioides* as a reservoir of natural enemies of *C. virescens*. Second, during our field observations the larvae of *C. virescens* were found mostly feeding on flower buds of *T. cacalioides*, but also fed on open flowers and leaves. Thus the feeding activity of the larvae could be affecting both the reproductive success and the vegetative vigor of the shrub.

Trixis cacalioides is native to the coastal valleys of the Atacama Desert of northern Chile (Zöllner 1976, Katinas 1996), where it is also used by some native host-specialist Lepidoptera (Vargas 2011, Méndez-Abarca et al. 2014). Furthermore, the near threatened status has been recently proposed for this shrub in the study site (Gatica-Castro et al. 2015). Obviously, the use of *T. cacalioides* by the voracious larvae of *C. virescens* can be a threat for both the shrub and the native Lepidoptera species that depend exclusively on this plant for their food. Accordingly, the effect of this polyphagous pest on the populations of *T. cacalioides* and its associated host-specialist Lepidoptera should be assessed in further studies.

ACKNOWLEDGMENTS

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Bárbara Santos-Zamorano, *Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Casilla 6-D, Arica, Chile*; Wilson Huanca-Mamani, *Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Casilla 6-D, Arica, Chile*; Héctor A. Vargas (Corresponding Author), *Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Casilla 6-D, Arica, Chile. E-mail: havargas@uta.cl; lepvargas@gmail.com*